[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **232nd Meeting** *of the Biochemical Society was held at the British Postgraduate Medical School, Ducane Road, Hammersmith, W.* 12, *on Saturday,* 22 *January* 1944, *at* 11.15 *a.m., when the following communications and demonstrations were given:*

COMMUNICATIONS

Observations on the Correlation between Capillary Resistance and Ascorbic Acid in the Plasma.

By HAROLD SCARBOROUGH and E. GILCHRIST

The view is widely held at present that there is a positive correlation between tissue ascorbic acid levels and capillary resistance and that therefore a low capillary resistance is associated with, and indicative of, frank and so-called subclinical scurvy. A statistical investigation of data obtained in 214 simultaneous determinations of capillary resistance (negative pressure method of Scarborough*) and plasma ascorbic acid (method of Farmer & Abt† slightly modified) provides no support for this view. In each of three 'standard areas' the range of capillary resistance corresponding to five groups of gradually increasing ascorbic acid values was wide, but the range became smaller and the standard deviations less at higher plasma ascorbic acid levels. The upper limit of the range was progressively lower in succeeding groups with higher plasma ascorbic acid values. The mode and the mean in each group similarly decreased. The capillary resistance in a group of 60 subjects with plasma ascorbic acid levels below 0·31 mg./100 ml. was significantly lower than in another group of 48 with levels between 0·91 and 1·20 mg./100 ml., the difference between the means exceeding twice its standard error for capillary resistance values in two out of three standard areas. Over the whole series of 214 double observations the correlation coefficient exceeded twice its standard error (in all three standard areas) but was negative in sign. Therefore lower (not higher) levels of capillary resistance are found in subjects with greater concentrations of ascorbic acid in the plasma.

Since particular care was taken to exclude all subjects in whom other conditions influencing capillary resistance might have been operative, and all cases of scurvy, it is felt that these results have a general application.

* Scarborough, H. [1941]. *Edinb. Med. J.* **48**, 555.

† Farmer, C. J. & Abt, A. F. [1936]. *Proc. Soc. Exp. Biol., N.Y.*, **36**, 146.

Carotene and Lycopene in Rose Hips and other Fruits.

By F. C. JACOBY and F. WOKES

Rose hips have been previously reported to contain carotene and lycopene* and vitamin A activity of 60–100 i.u./g.† We have now estimated carotene and lycopene separately in 28 different varieties of rose hips (from Prof. Heslop Harrison and from the Royal Botanic Gardens, Kew), also in tomatoes and *Solanum Dulcamara* berries, by a combined phase separation and chromatographic method. The pigments were extracted by grinding with quartz, petrol ether and acetone, the acetone was removed by washing with water in a continuous flow apparatus designed by Dr Vernon Booth, xanthophylls were removed with diacetonol, and the carotenoids adsorbed on alumina and eluted with benzene. The benzene solutions of the separated carotene and lycopene examined in the Hilger Nutting Spectrophotometer gave maxima in agreement with those obtained

by ourselves and other workers on pure specimens. The carotene and lycopene contents of the flesh of the rose hips calculated from extinction coefficients obtained from Dr R. A. Morton‡ were 40–240 and 90–650 μg./g. respectively. One of the highest carotene contents was found in a hybrid of *Rosa dumetorum* and *R. mollis*, kindly supplied by Prof. Heslop Harrison. A biological assay in the Pharmaceutical Society's laboratories of a sample of dried rose-hip extract prepared from *R. dumetorum* and *R. canina* after 9 months' storage at room temperature indicated vitamin A activity of 44 i.u./g., which was in agreement with chemical assays.

* Karrer, P. & Widmer, R. [1928]. *Helv. Chim. Acta*, **11**, 751. Escher [1928]. *Helv. Chim. Acta*, **11**, 752. Kuhn, R. & Grundmann, Ch. [1934]. *Ber. dtsch. chem. Ges.* **67**, 1133. Wokes, Johnson, Organ & Jacoby [1943]. *Nature, Lond.*, **151**, 279.

† Svensson, E. [1936]. *Skand. Arch. Physiol.* **73**, 237.

‡ Morton, R. A. [1943]. Private communication.

The Production of Acetic, Propionic and Butyric Acids in the Alimentary Canal.

By J. Barcroft, R. A. McAnally and A. T. Phillipson

The fermentation of cellulose is known to occur in the large intestines of herbivorous and other animals, and the significance of this process may be even more widespread than is at present recognized. There is no information regarding the quantities of volatile acids formed from cellulose *in vivo*, and although these acids are known to occur in the ingesta of herbivorous animals there is equally little information regarding the quantities present.

In the four species investigated, with the exception of the sheep the concentration of volatile acid expressed as g. acetic acid per 100 g. dry matter is greatest in the caecum; in the sheep the concentration in the caecum is exceeded only by that found in the reticulum and rumen. Figures for the total quantities of volatile acids in the caecum and colon of the four species are as follows: sheep (weight 56–86 kg.), 7·9–9·1 g.; horse (weight approximately 458–662 kg.), 249–355 g.; pig (weight 19–21 kg.), 2·3–8·7 g.; rabbit (weight varying in region of 1 kg.), 0·21–0·67 g.

In the sheep an additional site of fermentation is found in the diverticula of the stomach, the rumen and reticulum, in which organs together the quantity of volatile acid present is from 26 to 91 g.

The concentration of volatile acid when expressed as g. acetic acid per 100 g. dry matter varies throughout the large intestine in much the same manner in all four of the species investigated. It is highest in the caecum and progressively falls as the ingesta pass caudally. The fall is sharper in the sheep and pig than in the horse and rabbit. The concentrations attained in the caeca of these animals were as follows: sheep, 3·6–4·3 g. %; horse, 2·6–10·9 g. %; pig, 4·2–5·2 g. %; rabbit, 0·9–4·3 g. %.

In the stomach the concentrations found were negligible in the horse and rabbit, but in the pig and particularly the sheep greater concentrations were attained: pig, 1·4–1·8 g. %; sheep, rumen and reticulum 6·0–9·7 g. %, omasum 1·1–2·1 g. %, abomasum 0·1–0·2 g. %.

The volatile acids disappear from the ingesta before they pass to the glandular portion of the stomach, the abomasum.

The concentration of volatile acids in the small intestines was negligible in all species with two individual exceptions; thus in one horse and in one rabbit a concentration of over 1 % was found. In all species, however, the concentration was slightly higher in the caudal third of the small intestine than in the oral two-thirds. The low concentration of volatile acid in the small intestine and the high concentration in the large intestine is a clear indication that these acids are produced in the latter organ.

Absorption of Acetic, Propionic and Butyric Acids from the Alimentary Canal.

By J. Barcroft, R. A. McAnally and A. T. Phillipson

1. The concentration of volatile acids in the blood leaving certain parts of the gut is, in many mammals, higher than that in the general circulation. This is true of the large intestine in the horse, pig, rabbit, sheep, and of the rumen, reticulum and to a less extent omasum of the sheep.

Volatile acids in terms of c.c. of N/100 *acetic per* 100 *c.c. of blood from alimentary canal and general circulation.* (*Average results: the figures after the animals indicate the number of experiments*)

	Carotid artery	Jugular vein	Stomach				Small intestine	Caecum	Colon
			Rumen	Reticulum	Omasum	Abomasum			
Pig (4)	4·5	4·0	7·0				11·0	21	—
Rabbit (4)	5·5	4·0	—				7·5	24	—
Pony (1)	7·0	—	—				5·0	—	44
Sheep (14)	5·5	4·8	36	36	19	5	3·3	22	

The highest value obtained for the volatile acids in any sample of blood, expressed in c.c. of N/100 volatile acid %, is 109, which came from a sample draining the under surface of a sheep's rumen.

2. Distillation curves indicate that acetic acid is present in both the rumen contents and in the blood draining the rumen, but the proportion of acids of higher molecular weight than acetic is greater in the rumen contents than in the blood. These findings have been confirmed by Elsden's chromatographic technique.

The same type of distillation curve is found for the material taken from the caecum of the sheep and the large intestine of the horse and pig, and the blood from the large intestine of the horse.

3. The study of the rate of absorption of sodium salts from the rumen of the sheep of acetic propionic and butyric acids shows that acetate is rapidly absorbed, propionate less so, while butyrate is slowly absorbed, judged by the concentration reached in the blood.

4. The quantity of volatile acid, calculated as acetic acid absorbed in an hour from the reticulum and rumen of the sheep (together), is estimated to vary from 1 to 5 g., but this value is regarded as minimal.

Convincing proof of the possibility of direct absorption from the rumen of the lambs was sought by the administration of radio-opaque material capable, if absorbed, of being secreted by the kidney. This is inserted into a rumen from which all other paths of exit have been barred by surgical treatment. Sodium ortho-iodo-hippurate* so administered is found by X-ray examination in considerable quantities in the bladder, and correspondingly, iodine in the urine. The lining of the rumen, like the skin, is of stratified epithelium, but unlike the skin the corneous layer is thin. The general mass of the epithelium is similar to the Malpighian layer of the skin which is of course permeable to water.

* Kindly sent us from the Glaxo Laboratories by A. L. Bacharach. We have to thank Prof. H. A. Harris for allowing us the facilities of his X-ray equipment and for the assistance of Mr Fozzard.

The Destruction of Acetic Acid by the Beating Heart. By J. BARCROFT, R. A. MCANALLY and A. T. PHILLIPSON

Perfusion of the mammalian (rabbits) heart with Ringer's solution containing sodium acetate indicates that acetate is destroyed in its passage through the coronary vessels on much the same scale as Locke and Rosenheim found sugar to disappear.

The average rate of destruction over the first 2 hr. of perfusion is 26 mg./hr. (four experiments), and the second 2 hr. period 23·0 mg./hr.

In one experiment carried out over 6 hr. the results were as follows:

2 hr. period at 30° C.	1	2	3
Destruction of acetate, mg./hr.	28	26	21
Heart beat	Good	Good	Not so good

The strength of acetate used in the above experiments was usually 134·4 mg. in 350 c.c. Ringer. In one it was 141 mg.

Controls were carried out as follows:

(1) The fluid was perfused through the apparatus as usual except that a piece of rubber tube of appropriate resistance was substituted for the heart. The loss of acetic acid was 4·5 (mg.)/hr. (two experiments).

(2) The heart was analyzed at the end of the experiment for acetic acid, no significant amount was found.

(3) In one heart which refused to beat, 14 mg./hr. was used in the first 2 hr.

Further experiments showed that the amount of residual glycogen in the heart was no greater after perfusion with 'acetate Ringer', than with Ringer's solution devoid of acetate, though the hearts perfused with Ringer containing glucose contained somewhat more residual glycogen than those perfused with glucose-free Ringer. By residual glycogen, is meant glycogen present when the heart at the end of a perfusion experiment had lost its power of beating.

Average glycogen percentage of heart tissue

	Residual glycogen after perfusion			
Unperfused	Ringer	Ringer + acetate	Ringer + glucose	Ringer + acetate + glucose
0·24	0·06	0·055	0·12	0·17

The communications just given amount to a complete metabolic scheme starting with cellulose and perhaps other carbohydrates carried out by organisms in the gut which engender volatile acids, principally acetic and propionic; these in turn are absorbed into the blood and disposed of in at least one organ, the beating heart. The proportion of carbohydrate metabolized in this way is in the herbivora important enough to make this scheme rank alongside other metabolic patterns.

Plasma Acid Phosphatase in Carcinoma of the Prostate and the Effect of Treatment with Stilboestrol. By JEAN WATKINSON, G. E. DELORY, E. J. KING and A. HADDOW

In confirmation of the work of Kutscher & Wolbergs,* and Gutman & Gutman,† an extremely active phosphatase has been found in the normal adult prostate, and in the blood plasma of patients suffering from carcinoma of the prostate with metastases in the bones. This phosphatase, unlike that of bone, kidney and intestine, has an acid pH optimum of 4·9. In the majority of cases of prostatic carcinoma which we have studied

there was a high level of acid phosphatase which returned to normal on treatment of the patients with stilboestrol. The clinical condition showed a marked improvement. Normal human semen contained enormous amounts of the enzyme, but very little was found in the semen of a case of eunuchoidism.

* Kutscher, W. & Wolbergs, H. [1935]. *Hoppe-Seyl. Z.* **236**, 237.
† Gutman, A. B. & Gutman, E. B. [1938]. *Proc. Soc. exp. Biol., N.Y.*, **39**, 529.

A Method for the Estimation of N-Methyl-Chloro-Nicotinamide in Urine. By R. A. COULSON, P. ELLINGER and M. HOLDEN

The Vitamin P Activity of some Fruits and Fruit Products. By A. L. BACHARACH and M. E. COATES

It has been possible to apply the guinea-pig capillary resistance method to the biological estimation of vitamin P in certain citrus fruits, in rose hips, in black currants, and also in concentrates and juices made on a commercial scale for each type of fruit. Citrus fruits are the richest source of vitamin P so far investigated, but distribution between peel and juice differs from that of vitamin C; no significant difference was found for vitamin P activity. There are indications that little vitamin P activity is lost in the processing of commercial concentrates, but that there is some diminution in activity during storage for several months, even in the refrigerator.

Chemical Estimation of Vitamin B_6 in Foodstuffs. By A. C. BOTTOMLEY (introduced by S. K. KON)

For extracting vitamin B_6 from the test material and removing interfering substances, the method of Bina, Thomas & Brown* proved satisfactory, the step of adsorption on 'Superfiltrol' and elution with 0·5% alcoholic NaOH being a notable improvement on earlier methods of purification. To this point their method was followed, except that, with products of high fat content, an extraction with ether was interpolated between the first centrifuging and the neutralization of the acid extract. Assay by the recommended diazo reaction, however, led to high results. Instead, the alcoholic eluate was acidified (pH 5–6) with dilute acetic acid, the alcohol was distilled off and the residue dissolved in water. Aliquots were then assayed by the method of Bird, Vandenbelt & Emmett,† involving adsorption on 'Superfiltrol' (rather more than the specified amount was needed) and simultaneous elution and colour development with 2·6-dichloroquinonechloroimide in *n*-butyl alcohol.

A parallel estimation was carried out using the same quantity of test material with added vitamin B_6. From the photometer readings of the two solutions the vitamin content of the material was obtained, with automatic correction for incomplete recovery.

The following values (μg. vitamin B_6/g.) were obtained, figures in parentheses being the results of biological tests on rats by Dr K. M. Henry and Dr S. K. Kon, using the method of Copping‡: dried brewer's yeast 33·6 (29·9), wheat germ 17·5 (13·5), spray-dried skim milk 4·1 (3·8), dried National wheatmeal bread 2·0 (2·1), dried potato 9·0 (9·2), a dried mixed diet 2·2 (2·5).

* Bina, A. F., Thomas, J. M. & Brown, E. B. [1943]. *J. biol. Chem.* **148**, 111.
† Bird, O. D., Vandenbelt, J. M. & Emmett, A. D. [1942]. *J. biol. Chem.* **142**, 317.
‡ Copping, A. M. [1943]. *Biochem. J.* **37**, 12.

The Mineral Content of the Lungs of Workers from the South Wales Coalfield. By E. J. KING and G. NAGELSCHMIDT

The lungs of 54 workers from the South Wales coalfield have been analyzed chemically for silica, alumina and coal. The minerals contained in the lungs have been isolated as residues, and in these the quartz, mica and kaolin have been determined by X-ray diffraction pattern analysis.

The amount of coal and the relative proportions of siliceous minerals found in the lungs reflect the nature of the employment in which the worker had engaged, e.g. collier, hard-header, etc.

No relation was found between the severity of the pathological lesions in the lungs and the concentration of either coal or kaolin. The concentrations of mica and quartz, on the other hand, ran more or less parallel with the degree of fibrosis observed. Reasons are advanced for dismissing mica and accepting quartz as the most probably significant aetiological agent in the production of the South Wales pneumokoniosis.

Serum Protein and Plasma Volume changes in Adrenal Insufficiency. By R. A. CLEGHORN, J. K. W. FERGUSON and M. I. HANNA (introduced by E. J. KING)

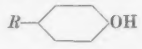
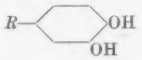
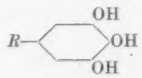
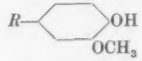
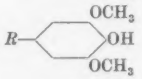
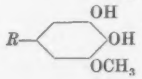
Formation of Haematins and Haemochromogens from Crystalline Haemin and Haemoglobin. By E. J. KING and G. E. DELORY

A Nutritional Survey among Pregnant Women. By J. YOUNG, E. J. WOOD and E. J. KING

DEMONSTRATIONS

Reaction of Anthocyanins with Molybdate. By H. BLASCHKO

When ammonium molybdate is added to solutions of anthocyanins in 1% HCl, colour changes are seen with those anthocyanins which contain free vicinal hydroxyl groups; no colour changes occur with anthocyanins not containing such groups. The colour changes are specific for the pigment examined. The reactions were observed on purified plant extracts; the results are summarized in the table:

Source of pigment	Anthocyanidin		Molybdate test
Geranium petals	Pelargonidin		–
Blackberries	Cyanidin		+
Corollae of *Campanula Portenschl.*	Delphinidin		+
Red peony petals	Peonidin		–
Petals of *Malva silvestris*	Malvidin		–
Berries of *Berberis Darwinii*	Petunidin		+

Some plants when grown in high concentrations of Mo show blue inclusion bodies in cells which normally contain anthocyanin;* under these conditions Mo seems to act as an inorganic co-pigment.

* Warington, K. [1937]. *Ann. appl. Biol.* **24**, 475.

Van Slyke's Copper Sulphate Method for measuring Specific Gravities of Whole Blood, Plasma and Serum. By R. H. A. PLIMMER

The older methods of determining the sp.gr. of blood with solutions of sodium sulphate or mixtures of organic solvents are not fully satisfactory. In this method drops of blood, plasma or serum are let fall into solutions of $CuSO_4$ of known sp.gr. Each drop becomes encased in a sack of copper proteinate and remains as a discrete drop without change of gravity for 10 or 15 sec., during which time its rise or fall shows its gravity relative to the solution. The size of the drop need not be constant, so a special pipette is not needed. Temperature correction is unnecessary, as the coefficients of expansion of $CuSO_4$ solutions approximate that of blood. The gravity need not be measured beyond $\pm 0{\cdot}0002$. The $CuSO_4$ solution cleans itself automatically after each test as the drop settles to the bottom, but must be renewed from time to time.

The standard $CuSO_4$ solutions are easily made from a saturated solution which is first diluted to a sp.gr. of 1·10 (or 159·62 g. $CuSO_4.5H2O$ per l. at 26·2°) and then using 1 ml. less than the last two figures of sp.gr., e.g. 1·073, i.e. 72 ml. and making to 100 ml.

The protein concentration is given by Moore and Van Slyke's formula $P=343\ (G_p-1{\cdot}0070)$ where G_p is the sp.gr. of the plasma, blood or serum, and P is g. protein/100 ml. A deduction of 0·0004 must be made from G_p when NH_4+K oxalate mixture is used to prevent clotting. Values are erroneous with lipaemic blood and in cases of N retention.

A Simple Step Photometer for Field and Emergency Use. By E. J. KING and G. E. DELORY

An inexpensive pocket-size instrument for all colorimetric analyses is easily made from a Hellige or Lovibond pH comparator. The nine coloured glasses are removed from an old disc with the aid of xylene. Ilford neutral grey screens ($\frac{1}{2}$ in.) are cemented in their places with Canada balsam. They should be of 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·7, 0·8 and 1·0 density, mounted in A. glass. Before the 2 holes in the comparator, through which the colours are normally viewed, is fixed a metal frame to hold the light filters (1×2 in.). Blue solutions are matched against the grey screens with the Ilford spectrum orange filter, red and brown with the spectrum green, and yellow with the spectrum violet. Coloured solutions are placed in the middle test-tube, and water in the left. The comparison is made in strong sun or electric light. The optical densities of a set of standards for each colorimetric method are determined by comparison with the grey screens, with the appropriate filter. The concentration of a coloured solution of unknown strength can then be established by determining its optical density in the same way.

A Case of Methaemoglobinaemia. By E. J. KING, MARGARET GILCHRIST and J. C. WHITE

The patient has suffered from methaemoglobinaemia all her life. The skin had a perceptible bluish tinge, most noticeable on the lips, ears and finger-nails. The condition was relieved by ascorbic acid. The blood findings were as follows:

Haemoglobin findings in a case of met-haemoglobinuria

			% Met. Hb		Blood count		Ascorbic acid		
Date	O_2 as g. Hb/ 100 ml.	Fe as g. Hb/ 100 ml.	Difference between O_2 and Fe	Spectroscopic	R.B.C. ($\times 10^6$)	Ret. %	Plasma mg./ 100 ml.	24 hr. urine (mg.)	W.B.C.* ($\times 10^3$)
24. viii. 43	10·80	17·0	36·5	33·0	—	—	0·34	—	—
1. ix. 43	10·95	17·0	35·6	32·7	5·3	2·6	0·31	1·37	10·0
2. ix. 43	Started ascorbic acid treatment—300 mg. per day								
3. ix. 43	11·23	16·44	31·7	32·3	5·5	—	0·38	1·36	8·0
8. ix. 43	14·07	—	—	13·9	5·9	1·3	1·17	56·5	6·0
15. ix. 43	14·25	15·85	10·1	11·8	5·1	—	1·16	106	11·0
23. ix. 43	14·8	16·1	9·8	—	5·6	0·2	1·36	59·2	—
1. x. 43	13·6	14·95	9·0	—	5·0	0·4	1·37	115	7·0
2. x. 43	Ascorbic acid doubled—600 mg. per day								
8. x. 43	14·2	15·3	7·2	—	5·0	<0·1	1·68	304	5·0
13. x. 43	13·4	14·8	9·5	—	5·1	<0·1	1·56	299	7·0
20. x. 43	13·1	14·4	9·0	—	5·1	0·4	1·45	419	8·0
27. x. 43	13·1	14·8	11·5	—	5·3	0·7	—	566	8·0
3. xi. 43	13·3	14·4	7·6	—	4·8	0·2	1·32	402	10·0
	Ascorbic acid treatment stopped								
10. xi. 43	12·8	14·7	12·9	11·7	4·7	0·3	0·61	8·6	5·0
17. xi. 43	12·1	15·0	19·3	18·3	5·1	2·2	0·47	9·9	10·0
24. xi. 43	11·7	15·3	23·6	24·5	5·6	2·0	0·34	—	16·0
1. xii. 43	11·8	15·8	25·3	26·7	5·0	0·8	0·35	16·9	5·0
15. xii. 43	10·8	15·8	31·6	—	4·7	1·0	0·23	9·3	13·0

* Differential white cell counts all normal: mean corpuscular volume = 90 cu. μ; packed cell volume = 49·5.

We are indebted to Prof. Henry Barcroft for securing this case for us.

The **Annual General Meeting** *of the Biochemical Society was held at the Courtauld Institute of Biochemistry, Middlesex Hospital, London, W. 1, on Saturday, 25 March* 1944, *at 2.30 p.m., when the following communications and demonstration were given:*

COMMUNICATIONS

Absence of Gross Displacement of Pantothenate and *p*-Aminobenzoate from Micro-organisms, by Pantoyltaurine and Sulphanilamide. By H. MCILWAIN

The reactions of certain micro-organisms to pantoyltaurine and to sulphanilamide are such that competitive interaction between pantoyltaurine and pantothenate, and between sulphanilamide and *p*-aminobenzoate, have been postulated and regarded as associated with displacement of the growth factors by the corresponding inhibitors. Such interaction has now been found to be a property of growing cultures in distinction to preformed cells.

Pantothenate was estimated in the presence of pantoyltaurine by growth of *Proteus morgani*, and examination of the solution and organisms after exposure of streptococci

to a large excess of pantoyltaurine showed that the inhibitor did not displace the growth factor from them. Similar observations were made with *p*-aminobenzoate and sulphanilamide in streptococcal and yeast cells, and are in agreement with the known existence of the two growth factors in cells in bound forms. The observed lack of action of the inhibitors upon preformed cells, coupled with their known effects upon cultures, is considered to be related to the bacteriostatic rather than bactericidal nature of their actions.

Partition Chromatography of Amino-acids. By R. CONSDEN, A. H. GORDON and A. J. P. MARTIN

It has been found possible to separate amino-acids on partition chromatograms using cellulose (paper) saturated with moisture as the stationary phase. *n*-Butyl alcohol, phenol, collidine and benzyl alcohol have all proved useful as mobile phases. The partition coefficients are close to those calculated on the basis of water content and ignoring the cellulose. The mechanical properties of paper have made possible two-dimensional separations, running first one solvent (say collidine) in one direction, drying the paper and running another solvent (say phenol) in a direction at right angles to that first used. The paper is then dried, sprayed with ninhydrin solution and heated, when the presence of the amino-acids is revealed by coloured spots. $\frac{1}{3}$ mg. of protein is sufficient. The presence of ammonia selectively slows the movement of aspartic and glutamic acids and speeds that of arginine, histidine, lysine and ornithine. Hydroxyamino-acids move slower than the corresponding amino-acids in phenol; but in collidine, the rates are similar. The presence of most of the amino-acids can be qualitatively demonstrated in a single experiment, and all can be shown by suitable variations.

Starch as a Medium for Partition Chromatography. By S. R. ELSDEN and R. L. M. SYNGE

In attempting to reproduce on a preparative scale the phenomena observed with cellulose (filter-paper) partition chromatograms by Consden, Gordon & Martin,* we have found washed raw potato starch a useful supporting substance for the aqueous phase.

With free amino-acids and peptides excellent separations can be obtained in starch chromatograms developed by butyl alcohol saturated with water. Separations have been studied by analysis of the effluent from the columns. For demonstration purposes the amino-acids are fixed and coloured *in situ* by passing a solution of ninhydrin in ether through the developed chromatogram, as suggested to us by Dr Consden.

With BuOH-H_2O chromatograms a variety of amino-acids and peptides are separated in the same order with starch as with cellulose, and it seems likely that this will prove true with other solvent systems.* So far the method has been used in isolative work; its quantitative applicability is not yet assessed.

* Consden, R., Gordon, A. H. & Martin, A. J. P. Accompanying communication.

The Ultra-rapid Examination of Milk for Chemical and Bacteriological Quality. (*a*) Single Quarter Samples at the Farm, (*b*) Herd Bulk Samples on the Creamery Platform. By J. G. DAVIS and S. J. WARD

(*a*) Since from an industrial point of view it is the chemical abnormality as such, rather than the presence of mastitis organisms, which is important, a quick and reliable method of assessing abnormality with which the dairy technologist or veterinary surgeon can examine a whole herd is urgently required. Of all biochemical abnormalities due to mastitis, the chloride content is the most suitable index, as (1) it can be measured very rapidly, (2) it is a reliable measure of chemical abnormality and hence suitability for

manufacture, and (3) it is unaffected by bacterial growth. A compact, portable apparatus has been designed which permits the measurement of chloride (by conductivity), and the detection of high pH values and clots, fibrin, etc., in 15 sec. The relation of conductivity to chloride content is linear except for 24 hr. colostrum (high globulin).

(*b*) A 15 sec. test is essential for creamery platform work. Although dye reduction tests are reliable, it was found that it required far longer than 15 sec. to attain a steady O/R potential in a churn of milk. The measurement of souring by glass electrode pH determination appears to be the only possible method for this purpose. Apart from the essential requirements of rapid working and robustness, troubles were experienced with film formation, stability of potentials and temperature errors. Various methods were tried to eradicate film trouble including high-pressure washing and wiping with a Sorbo rubber, but the only safe method appears to be the prevention of the formation of the film, which is an evaporative and/or aerobic phenomenon, by devising an electrode which is continuously in contact with a liquid. The ordinary temperature corrector is too slow for the present purpose, and this error has been avoided by drawing up the milk through a spiral in a thermostatically controlled bath at 25° C. The apparatus includes a conductivity meter, which not only permits the measurement of chemical abnormality in herd bulks but also allows an approximate correction for the 'mastitis effect', since mastitis raises the chloride appreciably and the pH slightly.

The Inhibition of Enzyme Systems by Metabolic Products of Carcinogenic Compounds. Urease and Succinoxidase. By L. A. Elson and C. Hoch-Ligeti

Kensler, Dexter & Rhoads* have shown that a number of aromatic diamines including *p*-phenylenediamine and dimethyl-*p*-phenylenediamine, which are metabolic products of the carcinogenic *p*-dimethylamino-azobenzene, inhibit the activity of yeast diphosphopyridine nucleotide and carboxylase enzyme systems. These authors, and Potter† who investigated the succinoxidase system of rat liver, and urease, conclude that the inhibition is due to the oxidation products of the diamines.

Elson & Warren have shown that when azobenzene is given to rats, benzidine can be isolated from the urine and evidence has been obtained that *p*-dimethylamino-azobenzene also undergoes the benzidine rearrangement in the body, one of the products of this change, 2:4-diamino-5-dimethylaminodiphenyl being excreted in the urine. We have therefore investigated the behaviour of 'benzidine rearrangement' products of this type on urease and succinoxidase enzymes which both contain —SH groups. Both were strongly inhibited.

The suggestion that the oxidation products are responsible for the inhibition has been confirmed, and in the succinoxidase system the rate of oxidation of the diamines appears to be related to the cytochrome present; in some instances inhibition was observed only after addition of cytochrome *C*. When the diamines are added to the complete system the rate of oxygen uptake is first increased, presumably due to the oxidation of the diamine by the cytochrome, and then inhibited, often completely. When the diamines were added before the substrate the initial increase could not be observed.

* Kensler, Dexter & Rhoads (1942). *Cancer Res.* **2**, 1.
† Kensler, Dexter & Rhoads (1942). *Cancer Res.* **2**, 688.

The Isolation of a Blood Group A substance from Pseudo-mucinous Ovarian Cyst Fluids. By H. K. King and W. T. J. Morgan

The quantitative* examination of 50 pseudo-mucinous ovarian cyst fluids has shown that these are frequently a rich source of the blood group substances. The procedure for the isolation of A-substance† from hog gastric mucin has been used and yields a

product similar in its chemical properties to hog mucin A-substance but significantly more active serologically as measured by the iso-agglutination inhibition test. Purified material obtained from a potent A-cyst fluid gave C, 44·8; H, 6·7; N, 6·0; CH_3CO 10 % and showed no reducing groups and only a trace of S. $[\alpha]_{5461} + 7°$. Relative viscosity, *Y*, 1·9 (C, 0·5 % in 1 % NaCl). Saline 1 %, *Y*, 1·00. Hydrolysis with N HCl at 100° gave 47 % reducing substances, 25 % hexosamine,‡ 4·4 % amino-N (Van Slyke) and 2·5 % α-amino acid N. Hydrolysis with 0·1 N Na_2CO_3 at 100° gave a maximum colour with Ehrlich's reagent after 10 minutes' heating equivalent to 15 % *N*-acetylglucosamine. Reduction (as glucose) likewise reaches a maximum 5·2 % in 15 minutes under the same conditions. If the products of alkaline hydrolysis are separated into diffusible and indiffusible fractions by dialysis through cellophane it is found that (1) the greater part of the material giving a colour with Ehrlich's reagent without further hydrolysis is diffusible, (2) most of the reducing substances are diffusible, (3) on complete acid hydrolysis of the two fractions amino-acids are yielded by the indiffusible fraction only whereas both hexosamine and reducing substances are liberated in about equal amounts from diffusible and indiffusible material.

* Morgan, W. T. J. & van Heyningen, R. (1944). *Brit. J. exp. Path.* (in the Press).
† Morgan, W. T. J. & King, H. K. (1943). *Biochem. J.* **37**, 640.
† Morgan, W. T. J. & Elson, A. L. (1934). *Biochem. J.* **28**, 988.

DEMONSTRATION

An Apparatus for Measuring the Abnormality of Single Quarter Milk Samples on the Farm. By J. G. Davis and S. J. Ward

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **235th Meeting** *of the Biochemical Society was held in the Medical School, Hospitals Centre, Birmingham* 15, *on Friday* 28 *July* 1944, *at* 2 *p.m., when the following communications and demonstration were given:*

COMMUNICATIONS

Effect of Vitamin B_1 on Histidine Excretion in Urine. By J. DAWSON (introduced by F. C. HAPPOLD)

Using the reaction described by Kapeller-Adler* the histidine excretion of normal man, pregnant women and women suffering from toxaemias of pregnancy have been examined. 89% normal subjects showed a histidine excretion of from 0 to 15 ml./100 ml. urine. In the case of pregnant women the histidine excretion is markedly increased as the pregnancy proceeds and is markedly reduced as pre-eclamptic toxaemia develops. This confirms Kapeller-Adler's findings.†

Under the influence of vitamin B_1 dosage 140 mg./day the histidine excretion of normal subjects disappears. Following the cessation of administration the histidine excretion returns. In the normal subject injected histidine is quantitatively excreted in the urine in 24 hr. Under the influence of thiamin dosage of 51 mg./day retention of injected histidine can be demonstrated. Following the cessation of thiamin administration some of the retained histidine is returned in the urine.

In cases of pre-eclamptic toxaemia the administration of thiamin reduces the urinary histidine excretion still further. Following the cessation of administration the histidine excretion rises to values held before thiamin was given. In one case in which the toxaemia appeared to have subsided, the low histidine excretion associated with thiamin administration was followed by a recrudescence of symptoms (cf. Kapeller-Adler, R. & Cartwright, J. A.).‡

* Kapeller-Adler, R. (1934). *Klin. Woch.* **13**, 21.
† Kapeller-Adler, R. (1941). *J. Obstet. Gynaec.* **43**, 141.
‡ Kapeller-Adler, R. & Cartwright, J. A. (1943). *Proc. Biochem. Soc.* **37**, ii.

The Action of Clavatin on Certain Enzyme Systems. By F. C. HAPPOLD and J. W. WATERS

The anti-biotic properties of Clavatin have been known for some time and have been described by several workers both in this country and in the United States. Following the observation of a certain amount of tissue degeneration during its use in chemotherapeutic experiments in these laboratories, the following investigations were carried out to shed some light on its mode of action.

Of the respiratory enzymes studied (both the Thunberg and the Barcroft techniques were employed), the following enzyme systems were completely (>95%) inhibited by 1·5 mg. Clavatin/ml.: glucose dehydrogenase, succinoxidase, malic acid dehydrogenase, α-glycerophosphate dehydrogenase, tryptophanase. The other oxidative enzymes tried were only partially inhibited. These were mannose dehydrogenase, lactic acid dehydrogenase, *d*-amino acid oxidase. Little effect was observed on uricase and cytochrome oxidase.

Other enzyme systems tried included the proteolytic enzymes trypsin and pepsin, pancreatic lipase, catalase, intestinal phosphatase, and vegetable tyrosinase. No inhibition whatever was observed on any of these systems.

With the exception of the tyrosinase, animal tissues, washed suspensions of *Bact. coli* or *Ps. Pycoyaneus* were used as sources of enzyme.

Further Observations on Growth Factors for *Lactobacillus casei* ϵ. By F. W. Chattaway, D. E. Dolby and F. C. Happold

In 1942 and 1943 we reported* on some of the properties of a growth factor for *L. casei* ϵ present in the amyl alcohol-insoluble fraction from liver extracts at pH 3. The active fraction was cresol-soluble and was precipitated as a silver salt at pH 7. We have recently reported† the presence of a further growth factor which does not influence the rate of acid production by *L. casei* ϵ, and that the diphtheria growth factor‡ is also a growth factor for *L. casei*. The original amyl alcohol-insoluble fraction has been split into two fractions, neither of which is folic acid, and there is some indication that growth factors also exist in the amyl alcohol-soluble layer.

These fractions are present in the filtrate after adsorption on fuller's earth at pH 3. It is adsorbed on norite and after elution is extracted with amyl alcohol. The aqueous layer is taken and extracted with phenol or *p*-cresol, the active fractions being soluble in the phenol at pH 3. The phenol-soluble fraction is saturated with baryta and the precipitate and filtrate separated. This process is repeated a further time with each fraction to give better separation. The precipitate was extracted with dilute sulphuric acid.

The following results constitute proof of separation:

Baryta precipitate fraction	1·0 ml.	3·6	ml. N/10 lactic acid (1 ml. ≡ 37·5 mg.)
	0·1 ml.	3·3	
	0·01 ml.	0·2	
	0·001 ml.	0·0	
Baryta soluble fraction	0·001 ml.	3·75 (1 ml. ≡ 215 mg.)	
	0·0001 ml.	1·35	
	0·00001 ml.	Nil	

0·01 ml. baryta precipitate + 0·00001 ml. baryta soluble gives 3·3 c.c.
" " + 0·000001 ml. " 2·3 c.c.

This last has a concentration of baryta soluble factor of 0·011γ/ml. medium.

* Chattaway, Happold, Lythgoe, Sandford & Todd (1942). *Proc. Biochem. Soc.* **36**, vi. Chattaway, Happold, Lythgoe, Sandford & Todd (1943). *Nature, Lond.*, **151**, 559.

† Dolby, Happold & Sandford (1944). *Nature, Lond.*, **153**, 619.

‡ Chattaway, Happold & Sandford (1944). *Biochem. J.* **38**, 111.

Isolation from Liver of New Factors Essential for the Growth of *Lactobacillus helveticus* and *Streptococcus Lactis* R. By E. C. Barton-Wright, W. B. Emery and F. A. Robinson

In an attempt to prepare a concentrate of the norite-eluate factor of Peterson *et al.* from a 70 % alcohol precipitate obtained as a by-product in the manufacture of a commercial liver extract (Examen), a fraction was obtained which markedly stimulated the growth of *Lactobacillus helveticus* when used in place of folic acid in the medium of Landy and Dicken.

The active fraction was treated with Decalso, and it was found that both the filtrate and the eluate obtained from the adsorbate by treatment with NaCl solution stimulated

growth. The activity was also precipitated on addition of silver nitrate at pH 7. By a procedure which included both these reactions a concentrate was obtained which stimulated the growth of *L. helveticus* and *Streptococcus lactis* when less than 50γ of solids were present.

Attempts were next made to concentrate the active factor further by partition chromatography according to the method of Martin and Synge, and attempts were therefore made to prepare a solution of the factor in some organic solvent. Rather unexpectedly treatment with chloroform gave an active extract, whilst the residual aqueous solution was also active. It was then discovered that an active preparation was obtained when Examen itself was extracted with chloroform.

For the present we propose to call the chloroform-soluble factor H.L. 1, and the chloroform-insoluble factor H.L. 2, since they stimulate the growth of both *L. helveticus* and *S. lactis*.

It was then decided to apply the fractionation procedure with Decalso to the chloroform extract of Examen, but the result was somewhat different from that obtained when the alcohol precipitate was similarly treated. The Decalso eluate was found to have only a slight growth-promoting activity for *L. helveticus*, though the filtrate was very active. When the two fractions were combined, however, growth as good as with the original chloroform extract, both with *L. helveticus* and *S. lactis* R., was obtained. The factors present in the eluate and filtrate are termed H.L. 3 and H.L. 4 respectively. They obviously differ from factor H.L. 1, which, although chloroform-soluble and adsorbable on Decalso like factor H.L. 3, was a very potent growth-stimulant, and from H.L. 2, which was chloroform-insoluble. The effect of factors H.L. 3 and H.L. 4 together is not additive, but synergistic.

None of the factors was destroyed by acetylation or benzoylation, whilst nitrous acid only affected factor H.L. 4, and although this was apparently destroyed, its synergistic effect with H.L. 3 was rather surprisingly not affected.

Neither of the chloroform-soluble factors H.L. 3 and H.L. 4 was affected when their aqueous solutions were boiled or when boiled with dilute acid or alkali. Thus, the factors differ in many respects from folic acid.

When the factors were tested on a Landy and Dicken medium in which the casein hydrolysate was replaced by a mixture of 16 amino-acids, appreciably less growth was obtained, suggesting that casein hydrolysate contains an essential growth factor or synergistic factor.

The chloroform-insoluble factor, factor H.L. 2, has now also been obtained from Examen by adsorption on charcoal or fuller's earth, and, like the chloroform-soluble factors, appears to differ from folic acid in being stable to acids and alkalis.

The Microbiological Assay of Nicotinic Acid. By E. C. Barton-Wright

The Nicotinic Acid Content of Cereals and other Foods. By E. C. Barton-Wright

The Effect of Mastication on Ascorbic Acid Content of Raw Vegetables. By G. N. Jenkins

Pyke's statement* that mastication of raw vegetables causes a considerable apparent fall in their ascorbic acid content has been confirmed. It has been found, however, that a similar fall occurs when cooked vegetables are chewed, which proves that ascorbic oxidase is not responsible for the effect. The mechanism of the apparent loss has been

analyzed and appears to be partly caused by an oxidizing agent present in saliva which oxidizes ascorbic acid to dehydro-ascorbic acid after the chewed vegetable is put into, and allowed to stand with, metaphosphoric acid. The only constituent of saliva which has been shown to produce this result is nitrite. Both saliva and nitrite bring about this change *in vitro* in acid media, but neither works at the normal pH of the mouth. It is unlikely, therefore, that oxidation of ascorbic acid occurs during mastication, though in the stomach the pH may be sufficiently low to favour this reversible oxidation by saliva.

Absorption from the mouth probably contributes to a small extent to the apparent loss during mastication but neither this, nor the oxidation to dehydro-ascorbic acid, will alter the antiscorbutic effect of vegetables, raw or cooked.

* Pyke (1944). *Proc. Nutrit. Soc.* **1**, 95.

A Crystalline Mucoprotein with High Choline Esterase Activity. By R. Bader, F. Schütz and M. Stacey

The Formation of Lower Glycerides during the Hydrolysis of Triglyceride with Pancreatic Lipase. By A. C. Frazer* and H. G. Sammons†

The formation of lower glycerides during lipolysis is known to occur in hydrolysis with acid or ricinus lipase.‡ It has not been reported as a stage in pancreatic lipolysis. In a series of 58 experiments on the hydrolysis of olive oil with pancreatic lipase, we have studied the end-products up to a 4 hr. experimental period. It is not possible to demonstrate free glycerol corresponding to the fatty acid which has been liberated. The acetyl value of the original triglyceride was 4, but after hydrolysis the acetyl value of the glyceride fraction had risen to 64. The effect of pH on the formation of lower glycerides runs parallel with the effect on the potency of the lipase. The change in acetyl value found indicates that 20–25 % of the glycerides are lower glycerides (calculated as monoglyceride).

This formation of lower glycerides during pancreatic lipolysis may be significant since it can be shown that a triple complex of bile salt/fatty acid/monoglyceride provides an emulsifying system for neutral fat which is not affected by changes of pH over a range of 5·0–9·0. With this complex emulsification is spontaneous and the size of the fat particles is of the order of 0·5μ or less.§

* Sir Halley Stewart Research Fellow.

† Lady Scott Moncrieff Research Scholar.

‡ Armstrong, H. E. & Gosney, H. W. (1914). *Proc. Roy. Soc.* B, **88**, 176. Grün, A. & Corelli, O. (1912). *J. chem. Soc.* p. 409.

§ Elkes, J. J., Frazer, A. C., Schulman, J. H. & Stewart, H. C. (1943). *J. Physiol.* **103**, 6*P*.

On the Temperature Factor in Adsorption on the Solid-liquid Interface. By R. Bader and F. Schütz

Solid adsorbents should retain more at low temperatures, but the speed with which equilibrium is attained is then low. Adsorption is therefore usually more successful at higher temperatures (e.g. room temperature), where the impact of the molecules is more frequent. During attempts of purifying enzymes by adsorption of these enzymes or impurities on different solids a procedure has been found advantageous which consists mainly in prolonging the contact of the adsorbent with the liquid over a period of many days, even weeks, at 0° C.

The Chemistry of Gram-staining. By H. HENRY (introduced by M. STACEY)

Sixty years ago, Christian Gram, a Dane working in Berlin, described a method of staining micro-organisms which differentiated these into two well-defined groups. The method consists in the use of a dye of the pararosaniline series followed by Lugol's iodine. The preparation is then washed in alcohol until no more of the blue dye appears in the wash, and is finally treated with a counterstain such as Bismarck brown, fuchsin, or neutral red. Organisms which retain the blue dye after alcohol extraction are designated as Gram-positive, whereas those which are decolorized by alcohol and take on the counterstain are termed Gram-negative.

These differences in staining characteristics correlated as they are with differences in behaviour to various reagents including bacteriostatics, suggest the existence of chemical and physical differential features of fundamental importance.

By suspending certain Gram-positive organisms in bile salt at 60°C. it has been found possible to separate from these an essential constituent of the Gram-positive molecular complex, leaving behind intact cells which are Gram-negative. Furthermore, it has been found that the extracted cells will after suitable reduction couple up with extracts so as to be Gram-positive once more.

The material which is common to all bile salt extracts is the magnesium salt of ribonucleic acid. So that the Gram-positive molecular complex would appear to consist of a combination of this salt with a basic protein.

This protein has now been separated from some Gram-positives and has been found to combine with the magnesium salt of ribonucleic acid forming a complex nucleoprotein which stains Gram-positive.

The Chemistry of Gum Tragacanth. By S. P. JAMES and F. SMITH (introduced by M. STACEY)

Gum tragacanth, an exudation of shrubs belonging to the genus *Astragulus*, has been shown to be a mixture of tragacanthic acid (*A*), a neutral polysaccharide (*B*), and a third component (*C*), the last being present in small amount. This conclusion was reached as a result of an examination of the product derived from the gum by exhaustive methylation when there were obtained the methylated derivatives of *A*, *B*, and *C* which were easily separable.

Hydrolytic cleavage of methylated tragacanthic acid with methyl alcoholic hydrogen chloride and subsequent fractionation of the mixture of methylated glycosides and ester uronosides formed, revealed the presence of 2:3:4-trimethyl α-methyl-*l*-fucoside, 2:3:4-trimethyl methyl-*d*-xyloside, 3:4-dimethyl methyl-*d*-xyloside, the methyl ester of 2:3-dimethyl methyl galactofururonoside and a monomethyl β-methyl galactopyrurononside, the relative proportions of which have been roughly estimated. The highly complex nature of the mixture of the cleavage products coupled with the difficulty of their separation does not allow a unique structural solution to be advanced but it is suggested that the galacturonic acid residues are present in the pyranose form as in pectic acid. This would mean that the structure of tragacanthic acid is essentially a chain of galacturonic acid residues mutually joined by 1:4-α links; the units which give rise to fully methylated fucose and xylose are attached to the main polygalacturonic acid structure through their reducing groups while the xylose units which give rise to the 3:4-dimethyl derivative may be situated between two acid groups of the main chain or interposed between the latter and the fucose or xylose 'end' residues.

The neutral methylated polysaccharide (*B*) was smoothly hydrolyzed by methyl alcoholic hydrogen chloride. Separation of the resulting mixture of methyl glycosides

by fractional distillation demonstrated the presence of 2:3:5-trimethyl methyl-*l*-arabofuranoside, 2:3-dimethyl methyl-*l*-arabopyranoside, β-methyl-*l*-arabopyranoside and a dimethyl hexoside. The high negative rotation of the methylated polysaccharide, together with its ease of hydrolysis, lends support to the view that the arabinose units are of the furanose type. However, the isolation of the dimethyl hexose shows that the polysaccharide is not a simple araban, a view also supported by the isolation of β-methyl-*l*-arabopyranoside as one of the cleavage products.

The third component (*C*) appears to be a steroid glycoside.

Observations on Complex Carbohydrates having Blood-group Specificity. By H. G. Bray, H. Henry and M. Stacey

Blood group A factors which contain carbohydrate and amino-acids have been isolated from a variety of sources including gastric mucin, pepsin, pancreatin, salivary mucin, frog spawn mucin, etc. All attempts to separate firmly bound residual amino-acid units from the carbohydrate portion result in a degradation of the complex with a corresponding loss in activity. Polysaccharides have been isolated from group A erythrocytes but these contain no amino-acid residues and have a very low activity. Landsteiner's idea that amino-acids play a role in determining serological specificity appears to be correct.

Blood group B factors have been isolated from salivary mucin and gastric mucin while complex polysaccharides have been isolated from gastric mucin of individuals in group O.

The carbohydrate residue of a group A factor from pepsin has been methylated and shown to contain derivatives of *l*-fucose (as end-groups), *N*-acetyl glucosamine, mannose and galactose. The molecule appears to be of the branched chain type of relatively low molecular weight.

DEMONSTRATION

Haemolymphatic Changes in the Lymph-nodes of the Rat, obtained (1) after Prolonged Administration and (2) shortly after a Single Injection of 1:2:5:6-dibenzanthracene. By A. Lasnitzki and D. L. Woodhouse

---

The **236th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Sheffield, on Friday,* 29 *September* 1944, *at* 11.00 *a.m., when the following papers were read:*

Fractionation of Fatty Acids and Bile Acids from Solutions of Crude Bile Salts by Adsorption on Foam. By R. Bader and F. Schütz.

While crystalline bile acids can be obtained by adsorption on foam,* as previously reported,† a preparation containing much fatty acids yields by foaming in the first foam fractions only crystalline fatty acids which, if once washed with water, give none of the usual reactions specific for sterols. This succeeds best at temperatures from 21 to 25° C. At lower temperatures the separation and yield becomes less good, while at higher temperatures no crystallization or separation was observed. After removal of the bulk of the admixed fatty acids by foam, pure bile acids start to crystallize on the foam, i.e. in the later foam fractions, which is marked by a sudden and obvious change in the foam

stability. The foam stability [foam time‡] of bile salts is greatly reduced by the presence of fatty acids, and, perhaps, other impurities. If the latter are foamed off the sudden increase in foam stability may be taken as an indication that mainly bile acids are then removed by the foam. Other factors influencing these results (pH, concentration, speed of 'foaming') will be briefly discussed.

* Schütz (1937). *Nature, Lond.*, **139**, 629. Schütz (1938). *C.R. Soc. Physiol. Suisse*, **46**.

† Bader & Schütz (1943). *Biochem. J.* **37**, *Proc.* ix. Bader & Schütz (1944). *Trans. Faraday Soc.* (in the Press).

‡ Schütz (1942). *Trans. Faraday Soc.* **38**, 85, 94.

The Sampling of Cooked Cabbage for Estimation of Ascorbic Acid in Nutrition Surveys. By H. G. Bray and W. V. Thorpe

During an investigation of the diet of hospital nurses and patients, an attempt was made to assess the loss of ascorbic acid which occurred in cabbage from the time when it was cooked to the time when it was consumed—a period varying from 15 min. up to 1 hr. Failure to obtain satisfactory duplicate analyses led to laboratory experiments in which cabbage was cooked and kept hot in a manner similar to that obtaining in the hospital. It was not found possible to obtain agreement between replicate samples. This was due to the impossibility of obtaining representative samples from the cabbage as served. Reference to the literature in which satisfactory analyses are given reveals the fact that the cabbage used was either minced or finely chopped. Under such conditions there is no difficulty in getting good agreement for replicate samples, but these ideal conditions do not usually apply in field work. In laboratory experiments in which cabbage was left as ordinarily served at table and not rendered homogeneous by mincing, values for ten multiplicate samples ranged, for example, from 32 to 75, from 25 to 78, from 11 to 56 mg. per 100 g. Random samples might be ± 70 % from the mean.

The Differentiation between Prostatic Phosphatase and other Acid Phosphatases in Pathological Human Sera. By Freda K. Herbert

In prostatic carcinoma an acid phosphatase derived from the tumour cells may pass into the blood, and the estimation of acid phosphatase in serum is an aid to diagnosis. Raised titres are almost constantly found when metastases are present, and are found also in a considerable proportion of cases without demonstrable metastases. In many instances the increases are so great as to be clearly diagnostic, but in some cases there are only slight increases, such as are occasionally found in other diseases. It is therefore of interest to distinguish the prostatic phosphatase from other acid phosphatases.

Preparations of phosphatase from the prostate have been made by grinding the finely cut tissue with sand, centrifuging the pulp, and taking off the supernatant fluid, which contains from 140,000 to 600,000 units of acid phosphatase. Experiments on two distinguishing properties of prostatic phosphatase have been made with these preparations in high dilution, with the prostatic enzyme added to serum, and with normal and pathological sera.

(1) *Destruction of the prostatic enzyme at* 37°. When serum containing prostatic phosphatase is incubated 1 hr. at 37° the prostatic enzyme is irreversibly inactivated. The acid phosphatase of normal serum is unaffected. Preparations of prostatic phosphatase are inactivated when the incubation is performed at pH 7·4; they are much more stable at pH 4·8.

(2) *Inactivation by alcohol at room temperature.* Serum, or an enzyme preparation, is treated with four-tenths its volume of ethyl alcohol, allowed to stand ½ hr. at room temperature, and samples taken for estimation of acid phosphatase. Under these conditions the activity of sera in various diseases other than prostatic carcinoma remains unchanged. Prostatic phosphatase in serum (whether in artificial mixtures or in pathological sera) is inactivated. The sensitivity to alcohol of the enzyme in prostatic tissue preparations depends upon the diluting medium, viz. (*a*) in water, little or no inactivation occurs; (*b*) in citrate or acetate buffer solutions, ranging from pH 4·8 to 7·4, the enzyme is inactivated; (*c*) in 0·9 % NaCl there is partial inactivation; (*d*) in a mixture of NaCl and $NaHCO_3$, comparable in concentration with serum, and of pH 7·9, inactivation is almost complete.

So far as present experience goes, alcohol inactivation gives better promise of specificity than inactivation at 37°. More work is required for an assessment of the diagnostic value of these differential tests.

Determination of Ascorbic Acid in Presence of Interfering Substances by the 'Continuous-Flow' Method. 1. Preliminary Control Tests. By L. J. HARRIS and L. W. MAPSON

Dehydrated vegetables, caramelized by exposure to unduly high temperatures during either processing or storage, may contain reducing agents of the nature of reductones, which, since they react rapidly with indophenol dye, will interfere in the chemical estimation of ascorbic acid if the ordinary method of direct titration is used. A procedure has been devised for the differential determination of both ascorbic acid and reductones present in such materials, and has been used satisfactorily for routine determinations. It depends on the use of an apparatus employing the principle of the continuous flow,* by which the complete time course of a reaction can be followed.

Control tests with mixtures of equal volumes of ascorbic acid (0·000228 M) and 2, 6-dichlorophenolindophenol (in equivalent strength) at pH 3·5 and temperature 20° indicate that after 0·28, 0·55, 0·78 and 1·1 sec. respectively, 64, 80, 84 and 87–89 % of the reaction is complete, whereas with 'gluco-reductone' (mixture of substances formed by heating a solution of glucose with alkali) in place of ascorbic acid the corresponding values are 26, 34, 37·5 and 38·5 %. With 'amino-reductone' (mixture of substances formed by heating a solution of glucose with glycine and alkali), they are 30, 38, 41 and 44 %. The values when plotted lie on well-separated smooth curves, and thus ascorbic acid can be readily distinguished from these interfering substances. Moreover, mixtures of gluco-reductone and ascorbic acid give curves in intermediate positions, in proportion to the relative amounts of each present; hence both substances can be accurately estimated when present simultaneously. In recovery tests, ascorbic acid and gluco-reductones could be determined under the above conditions with an error of less than 2 % of the total reducing value.

Other substances were examined in the same way and their velocity constants determined. Cystein and sulphite could be readily distinguished from ascorbic acid by their much slower rates of reaction, while dihydroxymaleic acid, thiosulphate, and the ferrous ion were distinctive in having relatively slow rates at pH 3·5 but much faster at pH 1·4. Reductic acid had half the velocity constant of ascorbic acid, while hydroxytetronic acid, alone of the substances examined, had approximately the same constant: there is, however, no evidence that either of these substances, or dihydroxymaleic acid, occur in natural products.

* Hartridge & Roughton (1923). *Proc. roy. Soc.* A, **104**, 395.

Determination of Ascorbic Acid in Presence of Interfering Substances by the 'Continuous-Flow' Method. 2. Tests with Dehydrated Vegetables, and with Various Raw and Cooked Fruits and Vegetables. By L. J. HARRIS and L. W. MAPSON

The reductants (interfering substances) formed when dehydrated vegetables are caramelized resembled gluco-reductones closely in their rate of reaction with 2, 6-dichlorophenol-indophenol at pH 3·5, and no indication was obtained of the presence of the other types of reductants mentioned in Part 1. Both the ascorbic acid and the interfering substance could therefore be estimated by the use of the 'continuous-flow' technique, the experimental evidence for this conclusion being as follows.

(1) Various dehydrated vegetables known to be devoid of antiscorbutic activity were heated for differing periods at 100° so as to cause caramelization to varying extents: application of the 'flow' test then accurately showed the presence of interfering substance and no ascorbic acid. (2) On the other hand, dehydrated vegetables which had known antiscorbutic activities, but had not been subjected to caramelization were shown by the test to contain ascorbic acid and no interfering substance. (3) Furthermore, in recovery tests, it was shown that a quantitative estimate was possible of the ascorbic acid or gluco-reductones, or both, added to various dehydrated vegetable preparations already containing either (*a*) ascorbic acid without the interfering substances produced on caramelization, or (*b*) the interfering substances without the ascorbic acid, or (*c*) both present simultaneously. (4) Results of determinations of ascorbic acid in caramelized dehydrated vegetables by the constant-flow method agreed well with those obtained by the formaldehyde method.*

The limit of error was low, the ascorbic acid or interfering substance, determined in recovery tests, ranging from about 97 to 103 % of the theory (expressed as a percentage of the total reducing content).

No significant amount of interfering substances was found in numerous fresh vegetables or in certain common fruit juices, nor in vegetables cooked by the usual methods of boiling or steaming, or processed or dehydrated in absence of caramelization. Hence, for the usual run of fruit and vegetable materials it is confirmed that the ordinary indophenol titration is accurate, since it gives the same result as that obtained by the 'constant-flow' method. Only with plant products heated in the presence of alkali, or heated to exceptionally high temperatures—as, for example, in the preparation of chip potatoes—are interfering substances encountered. In doubtful cases, or when new types of products are being examined, the flow method provides a rapid and convenient means of ascertaining whether or not certain interfering substances are present.

* Mapson (1943). *J. Soc. chem. Ind.* **62**, 223.

Diamidines as Inhibitors of Enzyme Action. By H. BLASCHKO and RUTH DUTHIE

The action of three diamidines in therapeutic use has been investigated: stilbamidine, pentamidine and propamidine. All three substances were found to have inhibitory actions on the diamine oxidase of pig's kidney (substrate used: M/200 cadaverine). The inhibitions were, in 10^{-4} M concentration, with stilbamidine 70 %; with pentamidine 33 % and with propamidine 27 %.

With preparations of monoamine oxidase from rabbit's liver (substrate used: M/100 tyramine) the inhibitions were much stronger with pentamidine and propamidine than with stilbamidine; in 10^{-4} M concentration pentamidine caused more than 90 % inhibition, propamidine 82 % and stilbamidine 21 %. With pentamidine there was still about 80 % inhibition at 10^{-5} M and about 30 % at 10^{-6} M.

These inhibitory effects appear to be specific; 10^{-3} M stilbamidine and pentamidine were without inhibitory action on *d*-amino acid oxidase or on l-cysteic acid decarboxylase.

The Nature of the Basic Proteins of Salmon Cell-Nuclei and its Bearing on the Chemical Composition of Chromosomes. By E. STEDMAN and ELLEN STEDMAN

The nuclei of all animal cells so far examined have been found to contain a basic protein which may be either of the protamine or histone type.* Of these two types, the former has been observed only in the spermatozoa of certain species of fish (e.g. salmon, herring), whereas the spermatozoa of other species of fish (e.g. cod) and nuclei of the somatic cells of mammals contain histones. In the absence of any definite evidence to the contrary, it might be concluded that the difference between protamine and histone is a species difference, each type of protein occurring in all the nuclei of the species in which it is found. That this view is incorrect is shown by the examination which we have made of certain somatic cells of the salmon. As first demonstrated by Miescher,† the sperm heads of this species contain as sole basic constituent a protamine (salmine). This result has been amply confirmed by others‡ and has again been verified in the present investigation. The basic protein which we have isolated from the nuclei from salmon erythrocytes and from those of salmon liver has, on the contrary, proved to be of the histone type.

This result has an important bearing on the nature of the chemical composition of chromosomes. Many authors§ have expressed the view that histones (or protamines) constitute the protein component of the chromosomes. If this were the case, the basic protein present in the nuclei of the somatic cells should be identical with that occurring in the nuclei of the generative cells. The lack of such identity, now demonstrated in the case of salmon, proves that histones and protamines do not enter in any essential manner into the structure of the chromosomes, and reinforces the evidence which we have already advanced‖ that the principal component of the chromosomes is the acidic protein, chromosomin.

* Stedman, E. & Stedman, E. (1943). *Nature, Lond.*, **152**, 556.

† Miescher, F. (1897). *Die histochemischen und physiologischen Arbeiten.* Leipzig: Vogel.

‡ See Kossel, A. (1928). *The Protamines and Histones.* London: Longmans.

§ E.g. Mirsky, A. E. & Pollister, A. W. (1943). *Biol. Symp.* **10**, 247. Darlington, C. D. (1942). *Nature, Lond.*, **149**, 66. Serra, J. A. (1943). *Bol. Soc. Broteriana*, **17**, 203.

‖ Stedman, E. (1944). *Edinb. Med. J.* (in the Press).

The Chemical Estimation of Vitamin E. By J. TOŠIĆ and T. MOORE

In an earlier paper* values were given for the vitamin E contents of various oils, as found by the $\alpha:\alpha'$-dipyridyl method of Emmerie & Engel.† The estimations were made on the non-saponifiable fraction of the oils, which were prepared with the addition of pyrogallol during saponification in order to prevent loss of vitamin by oxidation. No attempt was made to separate tocopherols from other reducing substances, but an allowance was made for the reducing action of carotenoid pigments.

For some oils, in particular tung oil, the values obtained by this technique seemed unduly high. Biological tests, however, have now indicated that tung oil has little or no biological activity. It is obvious, therefore, that some oils contain inactive reducing substances, other than carotenoid pigments, which must be removed before the $\alpha:\alpha'$-dipyridyl method can be successfully applied.

For this purpose an absorption column of acid-treated Floridin XS earth, as recommended by Emmerie & Engel‡ for the removal of carotenoids and vitamin A, was first tried. In benzene solution the reducing substances of tung oil behaved in the same way as α-tocopherol in passing readily through the column. A column of aluminium oxide, which had been freshly baked at 100° proved more satisfactory. This allowed the ready passage of α-tocopherol dissolved in petroleum ether containing 1 % of ethyl alcohol. Since carotene also passed readily through the column, a small correction was necessary with some natural oils. Of the reducing substances of tung oil, however, about 80 % were adsorbed by the column.

When the new technique was applied to the unsaponifiable matter of a specimen of wheat-germ oil it was found that about 40 % of the reducing substances were adsorbed, and 60 % passed through the column. The fraction which was not adsorbed proved active in biological tests, but no activity was observed at the level tested with the adsorbed fraction, after elution with petroleum ether containing 20 % of ethyl alcohol.

* Moore, T. & Tošić, J. (1943). *Biochem. J.* **36**, *Proc.* 10 July 1943, xiv.
† Emmerie, A. & Engel, C. (1938). *Rec. Trav. chim. Pays-Bas*, **57**, 1351.
‡ Emmerie, A. & Engel, C. (1939). *Rec. Trav. chim. Pays-Bas*, **58**, 283.

The Stabilization and Estimation of Pyruvic Acid in Blood Samples. By C. Long

The pyruvic acid concentration in whole blood changes appreciably after the blood is withdrawn from the vein; hitherto, therefore, it has been necessary to determine the blood pyruvate level very shortly after taking the blood sample.

The rise and fall of the blood pyruvate concentration in the presence of different anti-coagulants and enzyme inhibitors has been studied and can be explained in terms of the two following reactions:

(1) Phosphoglycerate $\rightleftharpoons$ phosphopyruvate $\rightarrow$ pyruvate.
(2) Pyruvate + triosephosphate $\rightleftharpoons$ lactate + phosphoglycerate.

The changes in the pyruvate level of blood samples can be prevented by collecting the blood in tubes containing a dried citrate buffer, pH 4·0, together with NaF and a suitable haemolytic agent. The determination of pyruvic acid in blood samples, thus treated, can then be carried out by the 2:4-dinitrophenyl-hydrazone method.*

* Lu, G. D. (1939). *Biochem. J.* **33**, 249. Bueding, E. & Wortis, H. (1940). *J. biol. Chem.* **133**, 585. Long, C. (1942). *Biochem. J.* **36**, 807.

The Antagonism of Some Antimalarial Drugs by Riboflavine. By J. Madinaveitia

The formal similarity between the formulae of Mepacrine and riboflavine suggests that the antimalarial action of Mepacrine may be due to an antagonism between the drug and one of the riboflavine-containing systems almost certainly present in the malaria parasite.

Since this parasite cannot be cultured outside the host's body and it is difficult to alter the riboflavine concentration of the tissues of vertebrate animals, the possible antagonism between Mepacrine (and other antimalarials) and riboflavine has been studied with reference to *Lactobacillus casei* ε. This organism has been selected for this work because it requires an extraneous supply of riboflavine, and culture media suitable for the purpose in view have been described in the literature.

With the technique devised it has been found that the action on *L. casei* of Mepacrine, quinine, Propamidine and methylene blue, which are known antimalarials, is inhibited by riboflavine. The action of a number of other bactericidal substances such as crystal violet, chloramine T, formalin, salicylic acid, mercuric chloride, penicillin and sodium fluoride is not affected by the concentration of riboflavine present in the medium.

Antibacterial Activity of some Antagonists of Pantothenic Acid. By J. Madinaveitia, A. R. Martin, F. L. Rose and G. Swain

McIlwain, Barnett and Robinson* have shown that many compounds structurally related to pantothenic acid possess bacteriostatic or bactericidal properties in vitro, due to a reversible antagonism of pantothenic acid, and with one of these compounds, pantoyltaurine, a therapeutic effect in infected rats has been demonstrated.

Other pantamides related to pantothenic acid have now been prepared, amongst them panthydrazide, and amides of dialkylaminoalkylamines and substituted aminoethylphenyl sulphones. Several exhibited marked antagonism towards pantothenic acid with respect to *Lactobacillus casei* ε and *Streptococcus haemolyticus*. Of those examined for therapeutic activity in rats infected with *Streptococcus haemolyticus*, only one, di-β-(N-pantoylaminoethyl)-*p*-aminophenyl sulphone, showed any activity and that but slight.

* McIlwain, H. (1942). *Biochem. J.* **36**, 417. McIlwain, H. & Hawking, F. (1943). *Lancet*, **1**, 449. Barnett, J. W. (1944). *J. chem. Soc.* p. 5. Barnett, J. W. & Robinson, F. A. (1942). *Biochem. J.* **36**, 357, 364. Barnett, J. W., Bupre, D. J., Holloway, B. J. & Robinson, F. A. (1944). *J. chem. Soc.* p. 94.

Antibacterial Activity of Substances related to 4-Aminobenzoic Acid. By A. R. MARTIN and F. L. ROSE

The current view of the mode of action of sulphanilamide drugs is that these substances interfere with the growth of bacteria by virtue of a competitive inhibition of 4-aminobenzoic, or some substance related thereto, which serves as an essential metabolite for the organisms. The introduction of nuclear substituents into 4-aminobenzoic acid in some cases leads to the production of antibacterial agents which appear to act by a similar mechanism. With other substituents the effect may be either to weaken the sulphanilamide inhibitory action of the parent amino acid, or to result in substances which are biologically inert. The most potent antibacterial compounds are the 3-chloro and 3-hydroxy-4-aminobenzoic acids. In the latter case a feeble but definite therapeutic action has been demonstrated in mice infected with haemolytic streptococci.

Metabolic Definition of a Drug-Receptor. By H. MCILWAIN

β-Haemolytic streptococci inactivated pantothenate (destroying its growth-promoting properties for the streptococci and for other organisms) at the rate of about 2 mμ mol./mg. dry weight/hr. while glycolyzing and while the concentration of pantothenate approximated to that of mammalian blood.

Of eleven compounds related to pantothenate, only four, which inhibited growth of the streptococci, inhibited their pantothenate metabolism. These included the chemotherapeutically active analogue, pantoyltaurine. Of bacterial species, five which synthesized pantothenate were insensitive to pantoyltaurine while six whose growth was inhibited by pantoyltaurine to varying degrees, required added pantothenate for growth, and were found to inactivate it. The concentrations of pantoyltaurine which were required to inhibit pantothenate catabolism in the different sensitive organisms, varied in the same manner as the concentrations required to inhibit their growth. The system or systems using pantothenate have thus the properties of pantoyltaurine receptors. They did not inactivate pantoyltaurine at a rate comparable to that of their reaction with pantothenate.

The concentrations of pantoyltaurine inhibiting growth or metabolism of different organisms, varied independently of the rate at which organisms inactivated pantothenate or performed the glycolysis which is associated with pantothenate catabolism. This applied also to a strain which had been trained to pantoyltaurine-resistance. There is thus no basis at present for considering the number of receptors to be greater in resistant than in sensitive bacteria, but the receptors or associated systems appear to differ qualitatively in the different organisms.

Observations on the Metabolism of Sulphamezathine in the Intact Body and in Isolated Tissues. By W. O. SYKES

When a single dose of sulphamezathine is given orally or parenterally to man, almost 100 % can be recovered from the urine, most of it (about 90 %) in the conjugated form. After oral administration of acetyl sulphamezathine about 1 % appeared in the urine as free sulphamezathine, the rest was recovered unchanged. The proportions of free and conjugated sulphamezathine were different in pigeons. After injection of the free form about 50 % of the excreted drug was free; after injection of the acetyl form up to 40 % of the recovered drug was in the free form. The free form also appears in the blood after injection of the acetyl compound; its concentration reaches 30 % of that of the conjugated form. Thus the pigeon is capable of hydrolyzing a considerable fraction of the conjugated compound. In sliced liver of guinea-pigs and rats conjugation was very small, never exceeding 10% of the added drug. On the other hand, up to 60 % of added sulphamezathine was conjugated in sliced or minced pigeon liver. Addition of pyruvate or fumarate had no effect on the rate of conjugation. About 30 % of added acetyl sulphamezathine is hydrolyzed in sliced or minced pigeon liver.

Metabolism of Acetoacetic Acid. By H. A. KREBS and L. V. EGGLESTON

Evidence is presented showing that acetoacetate undergoes the following two reactions in minced cardiac muscle:

(1) Acetoacetate + malate → β-hydroxybutyrate + oxaloacetate.
(2) Acetoacetate + α-ketoglutarate + H_2O → β-hydroxybutyrate + succinate + CO_2.

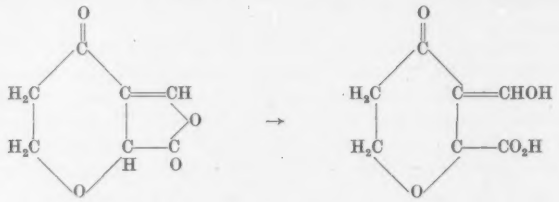
There are no indications that acetoacetate enters other reactions in minced heart. The results argue against the hypotheses of Breusch* and of Wieland & Rosenthal† according to which acetoacetate and oxaloacetate react to form citrate. It is true that acetoacetate may under some conditions increase the yields of citrate from oxaloacetate, but this can be explained by reaction (1): in the absence of acetoacetate some oxaloacetate is reduced to malate; reaction (1) regenerates oxaloacetate and makes it available for the reactions with pyruvate leading to citrate. The inhibitory effect of malonate‡, on the removal of acetoacetate is abolished by malate or other substances known to yield malate in the tissue. The malonate inhibition can therefore be explained on the assumption that malonate prevents the formation of malate required for reaction (1). The metabolic significance of the reduction of acetoacetate, and the mechanisms of the oxidative breakdown of acetoacetate must still be regarded as obscure.

* Breusch, F. L. (1943). *Science*, **97**, 490.
† Wieland, H. & Rosenthal, C. (1943). *Liebigs Ann.* **554**, 241.
‡ Jowett, M. & Quastel, J. H. (1935). *Biochem. J.* **29**, 2181. Quastel, J. H. & Wheatley, A. H. M. (1935). *Biochem. J.* **29**, 2773.

Enzymic Hydrolysis of Patulin. By H. A. KREBS

In the course of experiments on the effects of patulin* (claviformin,† clavacin‡) on enzymes, it was found that serum or extracts from liver, pancreas, and kidney convert patulin into an acid product. One molecule of patulin yields two equivalents of acid. According to Raistrick *et al.** treatment of patulin with alkali likewise produces two

equivalents of acid per molecule. They suggest that the formation of the acid is due to the hydrolysis of the lactone ring of patulin:



(patulin)

The enzymic conversion of patulin into an acid is most likely due to the same reaction. This reaction is somewhat unexpected since γ-lactones have previously been found to be stable towards enzymes. According to Bamann & Schmeller§ γ-butyrolactone, coumarin and santonin are not attacked by liver extracts. Lactoisocitric acid, tetronic acid and ascorbic acid are also stable towards hydrolyzing enzymes. Keeping serum for 2 hr. at 56° abolishes more than 90 % of the activity of the serum. Whilst patulin inhibits many enzymes, the product of hydrolysis is not a general inhibitor and does not prevent growth. The finding that certain fresh sera diminish the antibiotic action of patulin can at least in part be explained by the enzymic hydrolysis. Katzman *et al.*‡ found rabbit serum effective, but human and sheep serum ineffective. The enzymic activity of rabbit serum towards patulin is between 5 and 10 times greater than that of human or sheep serum.

* Raistrick, H., Birkenshaw, J. H., Bracken, A. & Michael, S. E. (1943). *Lancet*, **2**, 625.

† Chain, E., Florey, M. W. & Jennings, M. A. (1944). *Lancet*, **1**, 112. (1942). *Brit. J. Exp. Path.* **23**, 202.

‡ Katzman, P. A., Hays, E. E., Cain, C. K., Van Wyk, J. J., Reithel, F. J., Thayer, S. A., Doisy, E. A., Gaby, W. L., Carroll, C. J., Muir, R. D., Jones, L. R. & Wade, N. J. (1944). *J. biol. Chem.* **154**, 475.

§ Hoppe-Seyler (1931). *Hoppe-Seyl. Z.* **194**, 14.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **237th Meeting** *of the Biochemical Society was held at the National Institute for Medical Research, Hampstead, London, N.W. 3, on Friday, 24 November* 1944, *at* 2.15 *p.m., when the following communications and demonstrations were given:*

COMMUNICATIONS

Isolation of Ethanolamine from Acid Hydrolysates of Gramicidin. By R. L. M. Synge

Gramicidin was hydrolyzed with a mixture of equal volumes of 10N hydrochloric acid and glacial acetic acid for 24 hr. at 110–115°. After evaporation of the acid *in vacuo* the residue was dissolved in excess K_2CO_3 solution and subjected to azeotropic distillation under toluene at atmospheric pressure. The distillate was continuously circulated through a small volume of water before returning to the distilling vessel. Slightly impure 2-aminoethanol-1 was obtained in the water, representing rather more than 3% of the N of the various gramicidin specimens studied. Under the same conditions, a mixture of the known amino-acid components of gramicidin gave no volatile basic material, while 90% of added ethanolamine could be recovered from it.

The ethanolamine from gramicidin was identified by elementary analysis; titration of basic groups; determination of ammonia, acetaldehyde (none) and formaldehyde (2 mol.) liberated by periodate oxidation; determination of amino-N; optical inactivity.

Further work is required to ascertain whether the ethanolamine, so far only isolated by rather severe handling, is a true hydrolysis product or arises by dismutation. Neither is it yet decided whether the whole of the 'hydroxyamino-N' of gramicidin is present in this form. The facts here reported have interest in view of Hotchkiss's observations* on the specific power of gramicidin to inhibit bacterial metabolism of phosphate, and on the ability of cephalins (including phosphatidyl ethanolamine) to prevent this and the bacteriostatic action of gramicidin.

* Hotchkiss, R. D. (1944). *Advances in Enzymology*, **4**. New York: Interscience Publishers.

Retardation of the Growth of Mouse Carcinoma 2146 by Histone and Protamine. By E. Stedman, Ellen Stedman and F. W. Pettigrew

Partly on the basis of the observation that the nuclei of resting cells are characterized by a high, and those of proliferating cells by a low, content of basic protein, the view has been advanced* that histones and protamines (the basic proteins of cell-nuclei) possess the function of regulating mitosis, and this has led to the suggestion† that the transformation of normal into cancer cells is associated with, if not caused by, a diminution of the histone content of their nuclei. With the object of obtaining further evidence bearing on this hypothesis, the influence of histone and protamine on a transplantable tumour has now been examined.

The tumour employed is mouse carcinoma 2146, kindly provided by Dr P. R. Peacock. When 0·25 ml. of an emulsion of this tumour in saline is injected into the thigh muscle of a mouse, a large tumour is consistently produced in 7 days. If, however, the emulsion is prepared in a 2% solution of histone in saline, each mouse thus receiving 5 mg. of histone with the emulsion, practically no growth is visible within this period. Nevertheless, the histone does not kill the tumour cells, for growth usually occurs during the second week following the transplantation when the tumour may attain in 14 days

the size reached by the controls in 7, although the growth has, in a number of experiments, been much less rapid than this. The origin of the histone is apparently immaterial, for similar results have been obtained with histone isolated from the thymus gland of the calf, from carcinoma 2146 itself, and from the livers of mice. Protamine from herring sperm exerts a similar effect. On the other hand, neither the dried proteins from human blood plasma nor egg albumin produce, under the same conditions, any detectable influence on the tumour, while arginine appears to cause, if anything, a slight acceleration of its growth.

As is evident from the above results, the inhibitory action of histone is, under the conditions of our experiments, only of a temporary nature, a fact which might find an explanation in the resistance which would be offered to the penetration of material of high molecular weight into the nucleus of a cell. Nevertheless, the results are, as a whole, clearly consistent with the hypothesis which they were designed to test, although an alternative explanation of the mechanism of the effect observed cannot be excluded.

* Stedman, E. & Stedman, E. (1943). *Nature, Lond.*, **152**, 556.
† Stedman, E. (1944). *Edinb. Med. J.* **51**, 353.

N-Acetylglucosaminidase in Commercial Taka-diastase Preparations. By C. RIMINGTON

Taka-diastase is prepared by cultivating *Aspergillus oryzae* upon rice or wheat bran. The mould is collected and extracted providing a very active source of diastase. These crude preparations undoubtedly contain other enzymes.

N-Acetylglucosaminidase was prepared from the gut and liver of the snail *Helix pomatia* by Neuberger and Pitt Rivers and reported to have an optimum pH of 3·6 when hydrolyzing β-phenyl-N-acetylglucosaminide to phenol and N-acetylglucosamine.

In connexion with other experiments in progress, it was highly desirable to have a readily available source of N-acetylglucosaminidase. An examination of taka-diastase has shown that a very potent enzyme is obtainable from commercial preparations (Parke-Davis). Almost complete separation from diastase can be accomplished by fractional precipitation with alcohol at suitable pH; salt fractionation is unsatisfactory. Accompanying the enzymes and indeed making up some 40 % of the crude preparation of taka-diastase, is a complex polysaccharide containing hexoses and pentoses from which it is exceedingly difficult to free them by chemical means.

The electrophoretic behaviour of different preparations is reported in the following communication.

N-Acetylglucosaminidase was found by Neuberger and Pitt Rivers to be adsorbed, although somewhat irregularly, by Hungarian bauxite. Trials with Merck's alumina and with aluminium hydroxides A and C_γ showed that all adsorb the enzyme and to some extent also the polysaccharide, but C_γ afforded the greatest purification. The buffer salt employed is of importance.

A method for quantitative assay of taka-glucosaminidase has been worked out. At the optimum pH of 6·5, the product of enzyme concentration and time required to hydrolyze 50 % of the substrate is approximately constant over a wide range. One glucosaminidase unit (ϕ) is defined as the quantity of enzyme which hydrolyzes 1 mg. of β-phenyl-N-acetylglucosaminide in 100 hr. at 37°. In practice, a digest is set up containing 2 mg. of substrate and free phenol is determined at intervals of 2, 4 and 6 hr.

The optimum pH of taka-diastase, using starch and the achromic end-point method, is 5·2.

Electrophoresis of Taka-diastase Preparations. By A. S. McFarlane and C. Rimington

Taka-diastase, which has been subjected to several precipitations with alcohol, rapidly digests cellophane but may be dialyzed to ionic equilibrium against phosphate buffer in a nitrocellulose tube. During the process some 40% of the total solids, of low nitrogen and high carbohydrate content and negligible diastatic activity, passes through the membrane. The amber-coloured solution inside the tube shows on electrophoresis three well-defined boundaries in each limb of the U-tube. Electrophoresis for 48 hr. in the large Tiselius U-tube provides 30 ml. of a 1% solution of coloured material in the anode limb and approximately the same of a colourless material in the cathode side. The former has a high diastatic activity, high nitrogen and low carbohydrate content, while the latter has much carbohydrate, little nitrogen and no diastase. These fractions have been concentrated, redialyzed and examined in the medium Tiselius U-tube. Three boundaries again appear in each limb but in altered proportions, suggesting that the original material is a binary mixture of diastase and a carbohydrate complex containing glucosamine. The extra boundaries on each side are motionless and their behaviour is consistent with the view that they represent δ and ϵ changes in ion concentrations. The possibility that some small part of these boundaries is due to a third unchanged component in the mixture is not, however, excluded. The purest diastase separated in the anode compartment of the medium U-tube has an activity of 4300 units, 13·75% nitrogen and 11·0% carbohydrate.

J. Bacteriol 49(5), 39. 1945 "The Conditions Controlling the production of hyaluronidase by micro-organisms grown in

Conditions Controlling the Production of Hyaluronidase by Different Types of Micro-organisms. By H. J. Rogers (Beit Memorial Research Fellow) simplified media (48)

A simplified basal medium has been devised in which, when suitably supplemented, various micro-organisms produce hyaluronidase. The most important single condition for the synthesis of large amounts of the enzyme by all the bacteria tested is adequate buffering of the medium. Sodium glycerophosphate in a concentration of 2·5% has proved satisfactory for this purpose. The production of hyaluronidase by *Cl. welchii* (two strains), *Streptococcus* Group C type 7 (one strain) and *Streptococcus* Group A type 4 (one strain), is almost exactly proportional to the concentration of crude hyaluronate added to the medium. Relatively small amounts of enzyme are, however, synthesized by the organisms in the simplified medium unsupplemented by hyaluronate, and the titre obtained remains unaltered by continued subculture. Three strains of staphylococci, on the other hand, produce relatively large amounts of enzyme on the unsupplemented simplified medium and show no adaptive response to the inclusion of hyaluronate. This lack of response is unlikely to be due to the absence of other factors in the simplified medium limiting enzyme synthesis, since similar negative results were obtained upon peptone media. The titre obtained from *Cl. septicum* grown in the simplified medium either in the presence or absence of hyaluronate is very low compared with that obtained upon an Evans peptone medium, whereas the titres of enzyme obtained from streptococci, *Cl. welchii* and staphylococci grown on the two types of media are comparable. The addition of a diffusate from Evans's peptone to the simplified medium markedly increases enzyme formation by *Cl. septicum*, but no marked adaptation to the presence of hyaluronate in any of the media used is demonstrable.

* Rogers, H. J. (1944). *J. Path. Bact.* **56**, 284.

Artificial Induction of Coat Coloration in the Albino Rat by Means of 9-Phenyl-5:6-benzo-*iso*-alloxazine. By A. Haddow, L. A. Elson, K. M. Rudall, G. M. Timmis and Miss E. M. F. Roe

Administration of 9-phenyl-5:6-benzo-*iso*-alloxazine (I) to albino rats (200–250 mg./kg. by intraperitoneal injection, or by feeding), produces an orange-yellow pigmentation in the coat, restricted to certain areas in the form of a pattern with characteristic symmetry. Since fresh zones of pigmentation appear in response to injections at intervals, the pattern form is not a genetic one but depends upon a fluctuating process. That the process involved concerns the growth of hair is indicated from specially designed experiments in normal rats: only limited regions of the coat are regenerating hair at any given moment, and these areas are distributed in a symmetrical arrangement, as a complex series of waves, similar to the pattern evoked by injection of the specific *iso*-alloxazine. Variety in the growth patterns is due to the varying rate of progress of the growth wave front.

After treatment of several grams of the coloured hair with glacial acetic acid, removal of the acid with water, and chromatography of a chloroform extract, the hair pigment was obtained as an orange-yellow fluorescing substance, the ultra-violet absorption spectrum of which shows the presence of the injected compound or of some simple derivative which is spectroscopically indistinguishable under the conditions employed. It is therefore likely that the coloration produced by I is due to localization in growing hair of the compound itself or of a derivative. The probable mechanism of such localization is shown by visualization of the capillary circulation to the hair follicles. The greatest density of capillaries is found round the advancing edge of the growth wave, so suggesting the means by which the wave progresses and by which the circulating pigment enters the growing hair. This interpretation is confirmed by capillary visualization in rats both sheared and injected with I, to relate capillary density, the position of the wave front, and the pigmented tracts, simultaneously.

To a lesser degree, coat coloration is also produced by the first five members of the homologous series of 9-alkyl-5:6-benzo-*iso*-alloxazines. Direct correlation has been obtained between intensity of coloration produced, and rate of elimination of a pigment, for the most part identical with the administered substance, in the faeces and urine. Hence intensity of coloration is in all probability correlated, within this class, with facility and degree of absorption of the compound injected. Among related substances, a slight degree of hair pigmentation is induced by 9-phenyl-*iso*-alloxazine.

The Chemical Conversion of Diiodotyrosine into Thyroxine. By C. R. Harington and Rosalind V. Pitt Rivers

Formation of thyroxine by incubation of diiodotyrosine for 14 days at 38° (cf. von Mutzenbecher, 1939)* does not occur at pH <7 or >13; the optimum is at pH 10. Addition of hydrogen peroxide shortens the reaction time to 15 hr. at 38° and to 1–2 hr. at 90°, the pH effect remaining the same. The best yields of thyroxine (about 4% calculated on diiodotyrosine destroyed) are obtained by oxidation with peroxide at 90°, the thyroxine being protected by extraction into butyl alcohol during the reaction [Leland & Foster, 1932]†.

The pH effect supports the theory advanced by Harington [1944]‡ that the reaction involves oxidation of diiodotyrosine as the phenoxide ion; it leads to the further suggestion that the effective oxidising agent is iodine which can exist as such in the range where thyroxine is formed but at pH >13 will be converted into hypoiodite.

This suggestion is supported by the successful oxidation of diiodotyrosine to thyroxine with iodine at pH 8·4 and 10.

Peroxide is probably an effective oxidising agent in so far as it liberates iodine from diiodotyrosine. Such liberation will itself be affected by pH since the stability of diiodotyrosine (as indicated by its behaviour on heating in aqueous solution) is profoundly influenced by this factor; the range of relative instability of diiodotyrosine includes the pH at which it can be oxidised to thyroxine.

* von Mutzenbecker, P. (1939). *Hoppe-Seyl. Z.* **261**, 253.
† Leland, J. P. & Foster, G. L. (1932). *J. biol. Chem.* **95**, 165.
‡ Harington, C. R. (1944). *J. chem. Soc.* p. 193.

The Lysine Requirement of the Adult Rat. By A. NEUBERGER and T. A. WEBSTER

DEMONSTRATIONS

Artificial Induction of Coat Coloration in the Albino Rat by Means of 9-Phenyl-5:6-benzo-*iso*-alloxazine. By A. HADDOW, L. A. ELSON, K. M. RUDALL, G. M. TIMMIS and Miss E. M. F. ROE

The Assay of Penicillin. By P. BRUCE WHITE (introduced by C. R. HARINGTON)

Technique of Preparative Electrophoresis with Large Tiselius U-Tube. By D. BUNN and A. S. MCFARLANE

PRINTED IN GREAT BRITAIN BY W. LEWIS, M.A., AT THE UNIVERSITY PRESS, CAMBRIDGE

C37
0585
1 32E
2 5E3
3 32E
4 283
5 582
6 E38